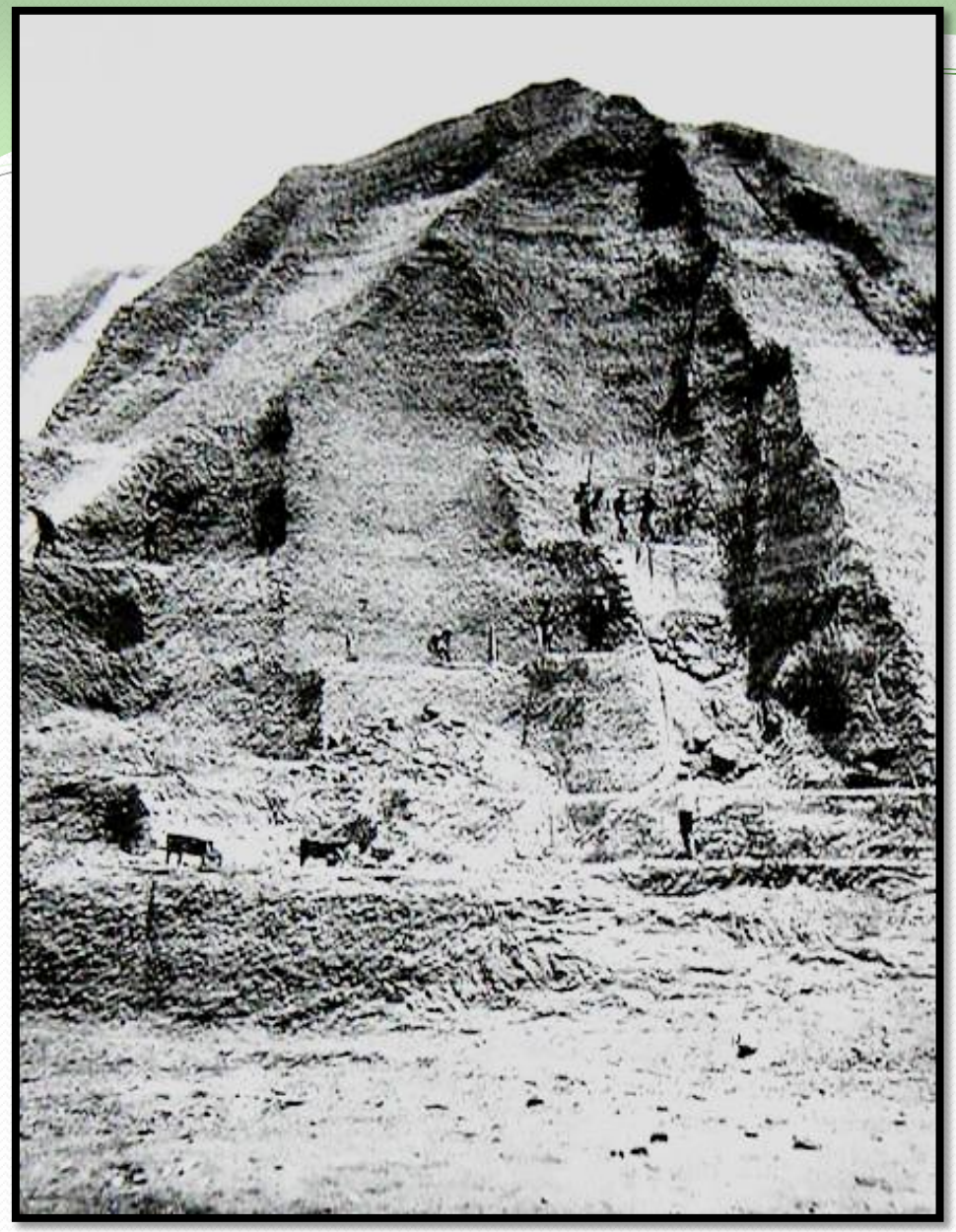
Microorganisms: One Stop Shopping for Chemicals for Any and All Agricultural Needs

George Lazarovits
A&L Biologicals, 2136 Jetstream Road, London, On. N5V 3P5
Lazarovitsg@alcanada.com , cell 519-878-1323

FOOD PRODUCTION IS THE BASIS OF CIVILIZATION

To produce large quantities of food we need:

1. **Productive land including the microorganisms**
2. **Fertilizers N-P(O4)-K plus trace chemicals Zn, Mn, Cu, Fe, Mg, Ca etc.**
3. **Plant protection from weeds, insects, fungi, bacteria and nematodes**
4. Quality seeds and good genetics
5. Ample and dependable supply of water



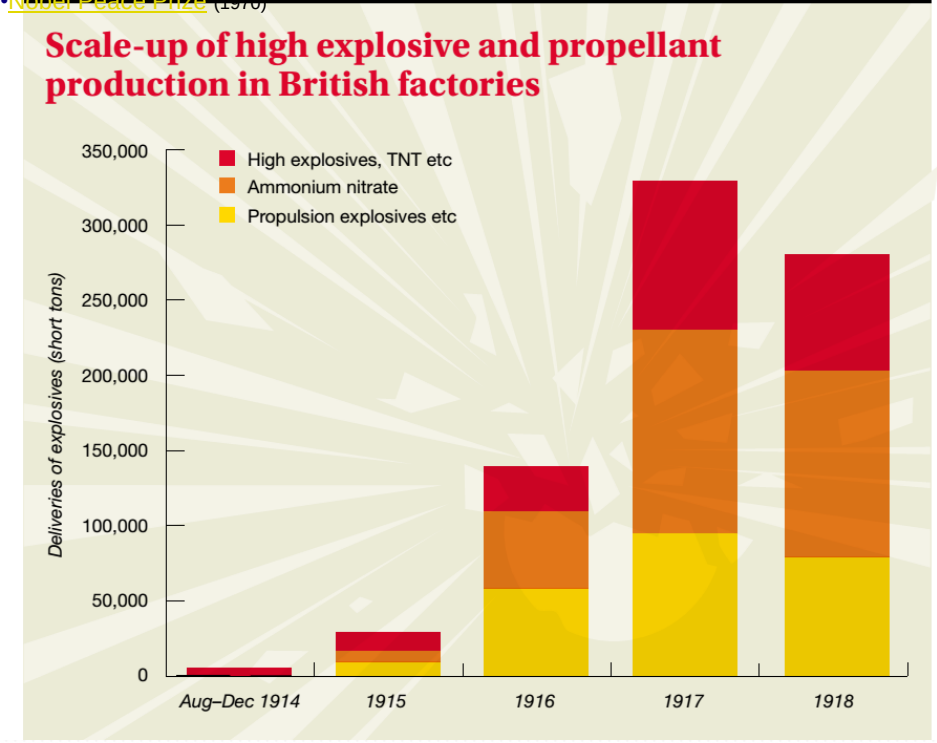
Mining guano in the Chincha Islands off the central coast of Peru. 1860.

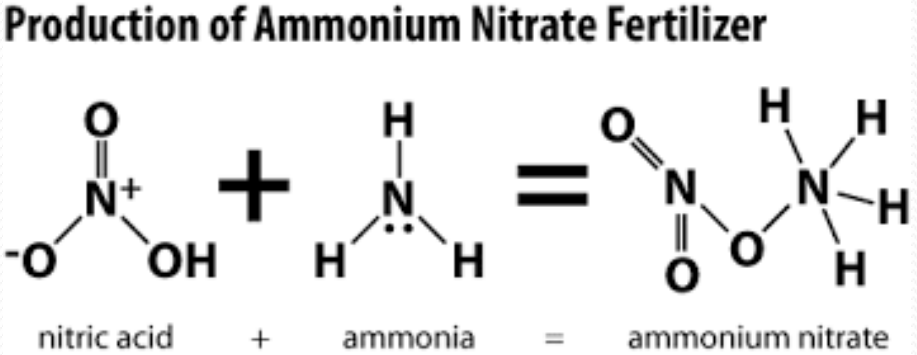
The First Green Revolution

- **Millions of fish eating birds + dry conditions off the islands of Peru provided ideal guano fertilizer**
- **Between 1840 - 1880, the Peruvians excavated over 20 million tons of guano for export = $2 billion in profits.**
- **By 1910 the reserves had become depleted and harvest became regulated**

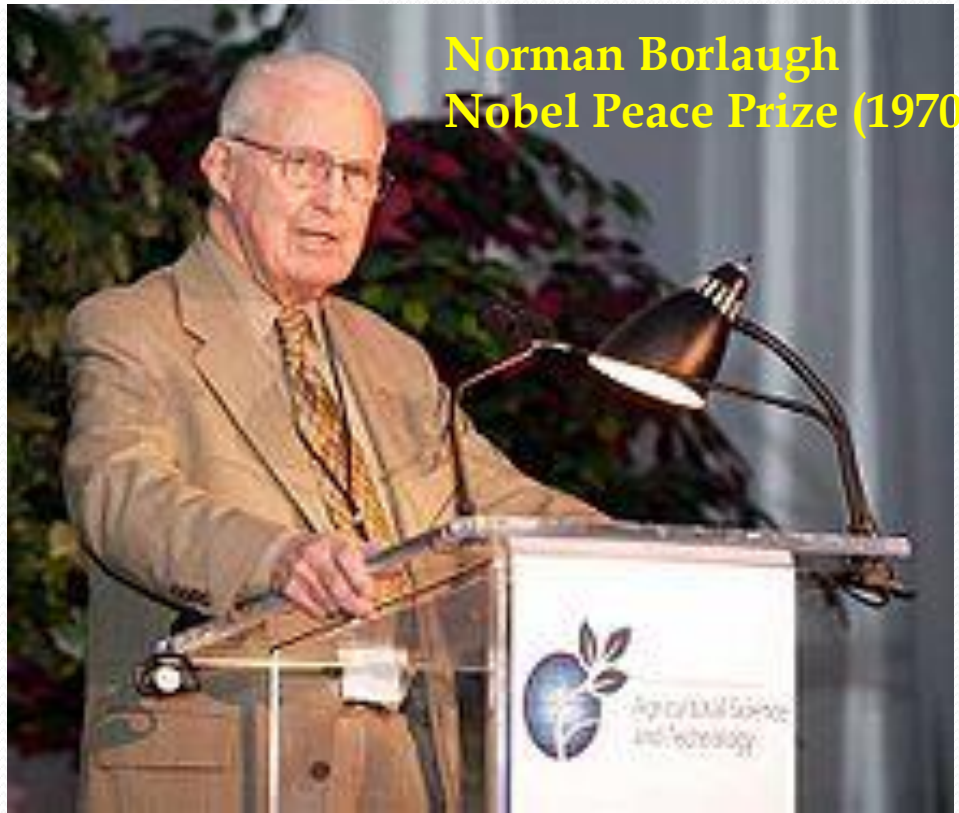
The Second Green Revolution

Greatly based on the Haber–Bosch nitrogen patents





Negro farmer hauling bags of dry fertilizer onto his truck, San Augustine, Texas, 1939. FSA.



Norman Borlaugh
Nobel Peace Prize (1970)

We have destroyed a third of Earth's farmland in 40 years

- Soil is being destroyed 100 X faster than it can form
- to avert disaster, farmers must adopt sustainable agricultural practices based on ecological principles.

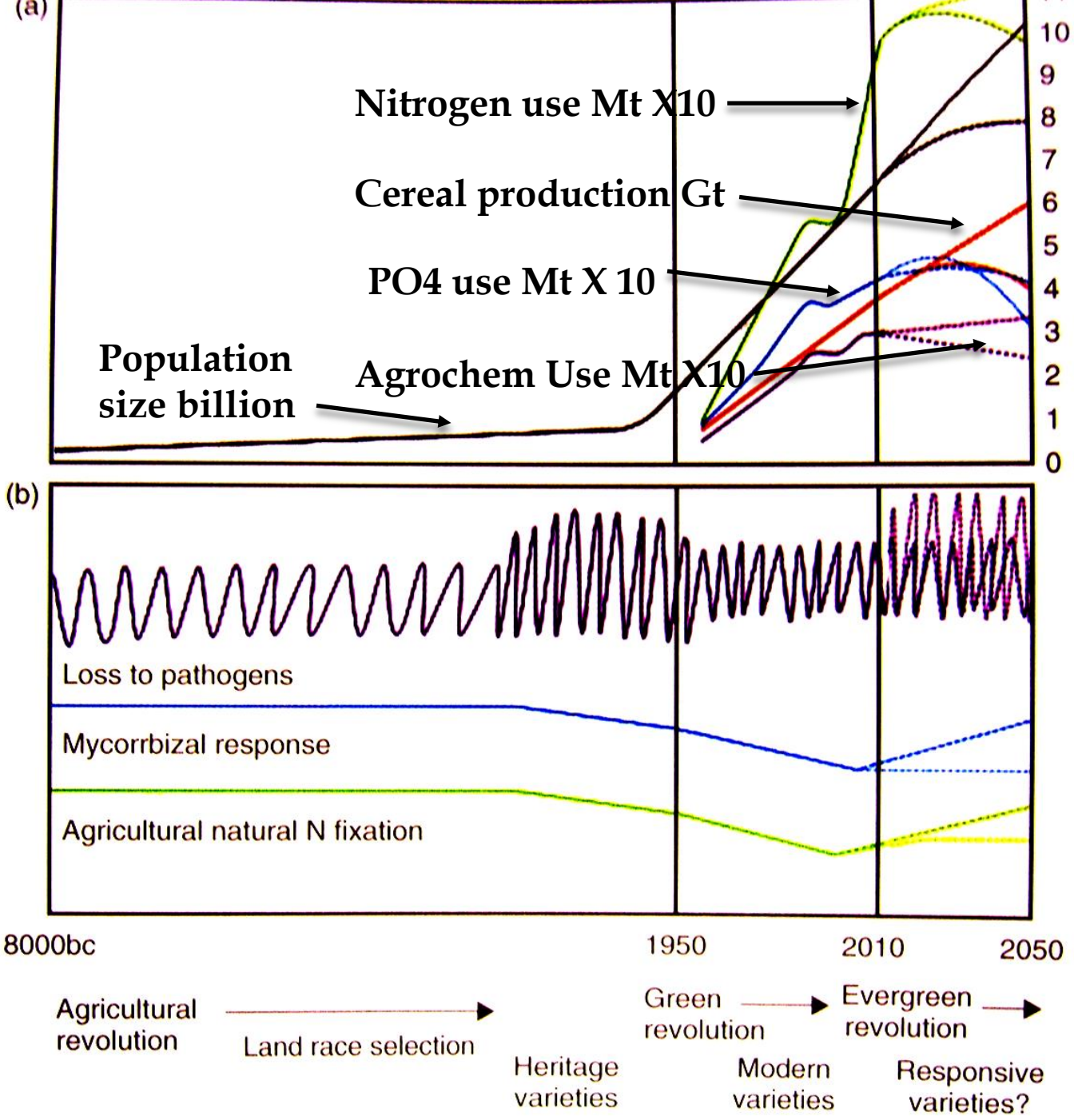
http://news.sciencemag.org/sifter
USDA NRCS SOUTH DAKOTA/FLICKR (CC BY-SA 2.0)

The New York Times

We Need a New Green Revolution

By PHILLIP A. SHARP and ALAN LESHNER JAN. 4, 2016

JANUARY 4, 2016



THE EVERGREEN REVOLUTION

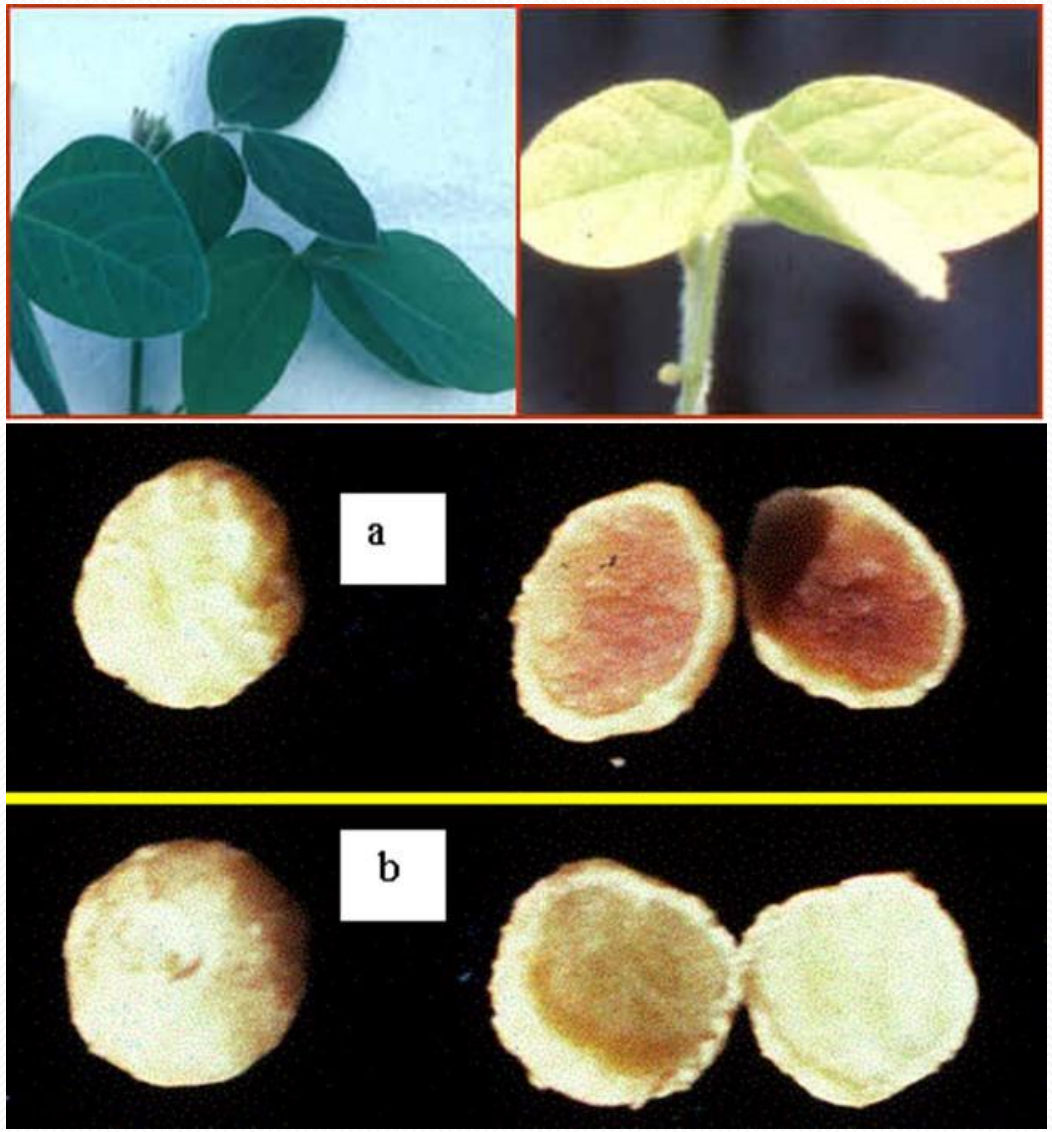
A.E. Bennett et al. 2013
Molecular Microbial Ecology of the Rhizosphere

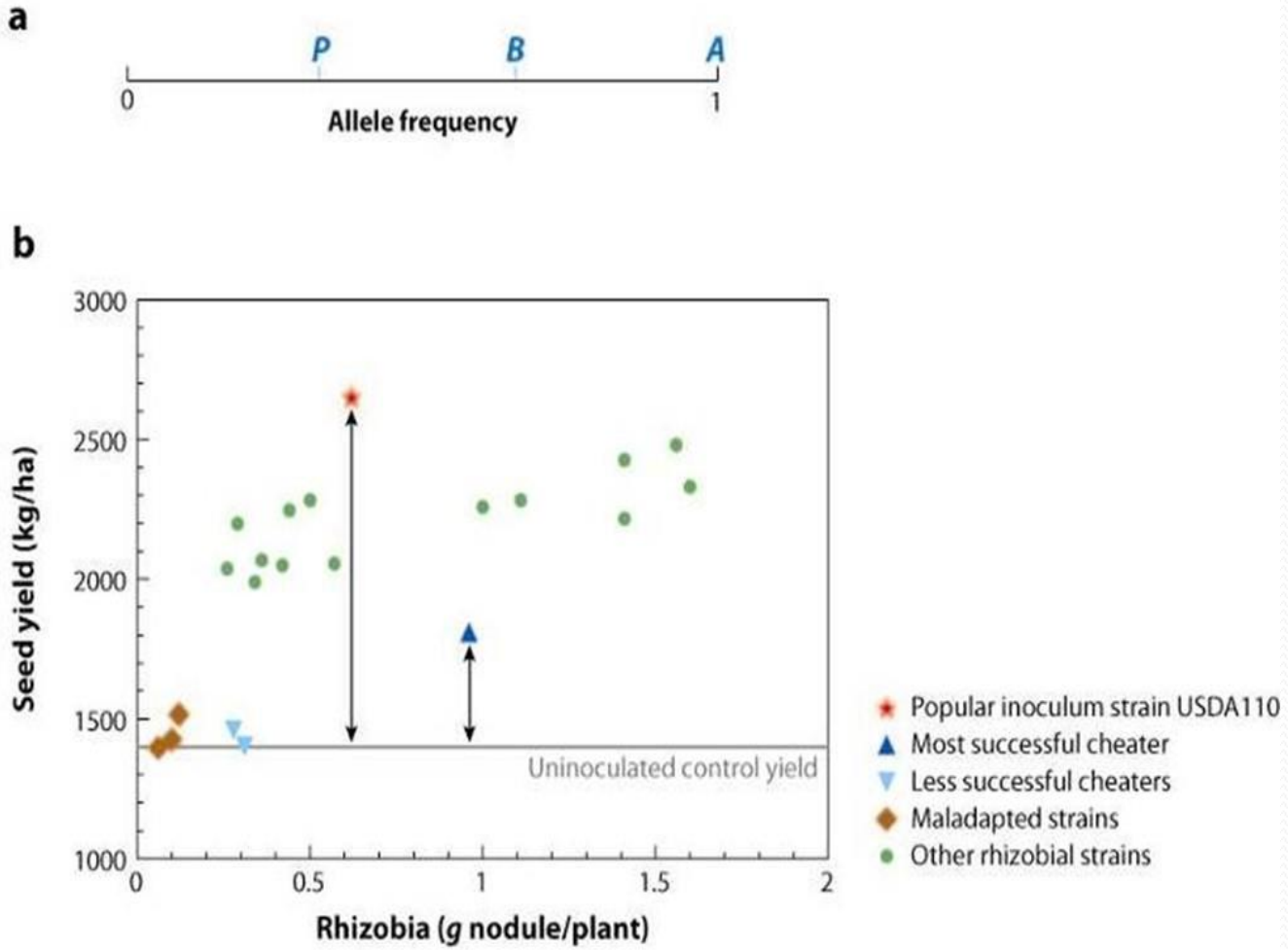
Agriculture based on ecology

Ecological agriculture

Practices that require greater reliance on natural soil processes, native microorganisms, and the interactions between plants, animals, and humans.

ARE ALL RHIZOBIUM INTERACTIONS BENEFICIAL?





Kiers ET, Denison RF. 2008.
Annu. Rev. Ecol. Evol. Syst. 39:215–36

Sugar cane endophytes were shown to be an integral component of production

Gluconacetobacter diazotrophicus, *Herbaspirillum spp.*, *Azospirillum* spp. and *Burkholderia* spp.

Table 3. Nitrogen fertilizer levels applied to sugarcane plants grown in different countries
Source: IFA (1999)

Country	Nitrogen fertilizer (kg ha^{-1})
Argentina	100
Australia	150–250
Brazil	50
India	100–300
Mexico	120–200
Philippines	120–200
South Africa	80–120
USA — Hawaii	300–400

BACTERIOCINS Annu. Rev. Genet. 1998. 32:255–78

- All bacteria produce antibiotics - you just need the right target organism to detect them

- *Agrobacterium radiobacter* K84, used worldwide for a 100+ years as a biocontrol of crown gall disease caused by *A. tumefaciens*, produces agrocin 84.
- Bacteriocins, peptide and protein antibiotics, kill closely related species through multiple modes of action
- Many consider these compounds to be too specific
- Potential of these compounds remains untapped

Antifungal Rhizobacteria represent 20-40% of all bacteria isolated from a crop plant

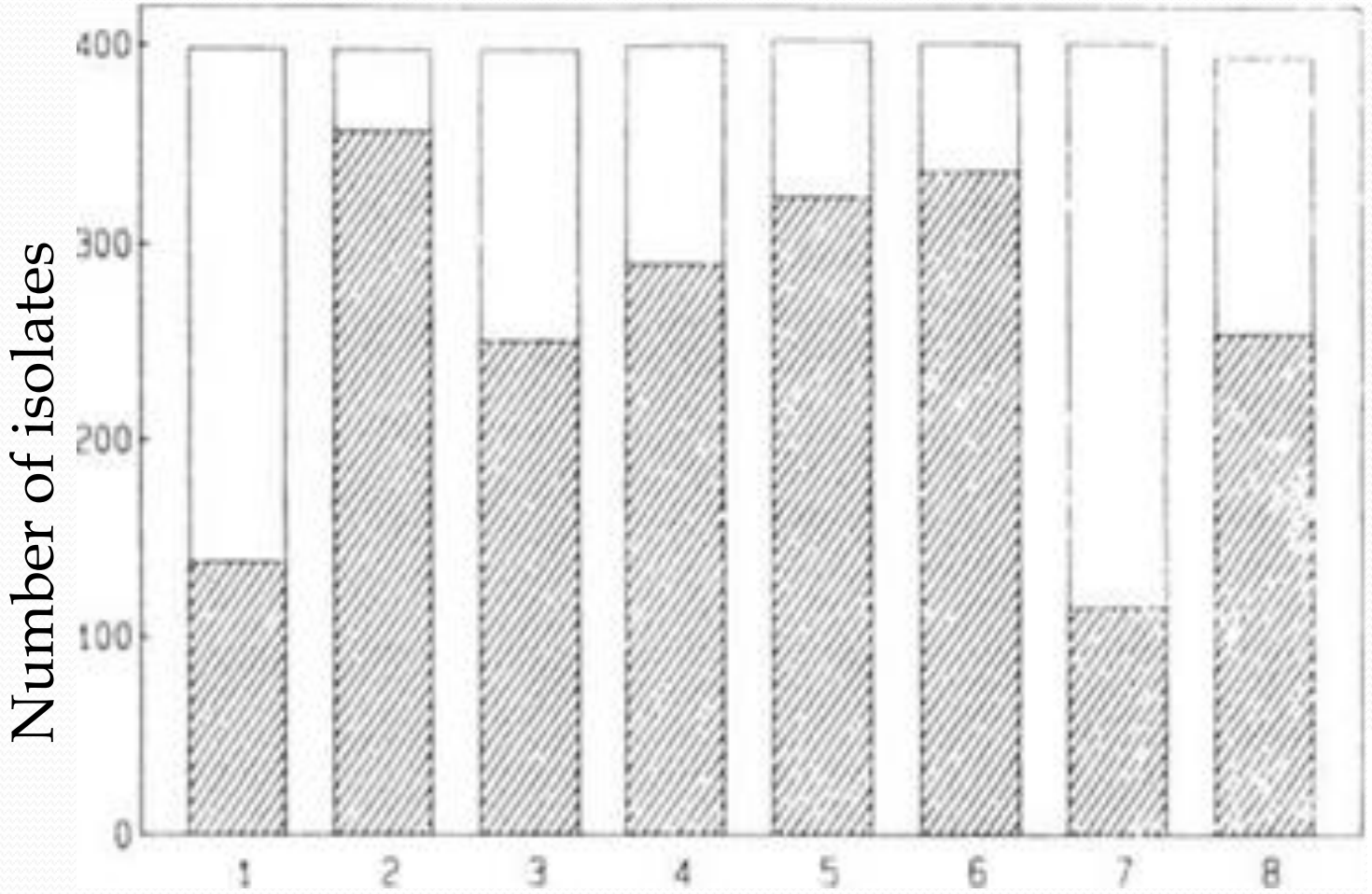
Crop	Plants examined	Isolates	Antifungal isolates
Sugar beet	1550	6780	2603
Corn	503	1508	403
Soybean	450	1139	244
Sunflower	450	1119	239
Barley	36	175	161
Chicory	22	662	24
Grapevine	36	231	231
Total	**3047**	**11614**	**3910**

Lievens et al 1989 Pestic Sci 27:141

Rhizobacteria from sugarbeet with activity against major fungal plant pathogens

1. *Pythium irregulare* 2. *P. betae* 3. *Rhizoctonia solani* 4. *Aphanomyces laevis,* 5. *Sclerotinia minor* 6. *Botrytis sp*. 7. *Fusarium oxysporum l. sp. pisi* 8. *Fusarium oxysporum f. sp. betae*

6780 strains tested
470 and 1746 active
1 & 2 respectively
Shaded boxes - active
Blank boxes – inactive



Lievens et al 1989 Pestic Sci 27:141

Microbial Fungicides in the Control of Plant Diseases

J. Phytopathology155, 641–653 (2007) BS Kim and BK Hwang

- Strobilurin fungicides derived from the fungus *Strobilurus tenacellus*
- Highly specific mode of action
- Very active but photochemically unstable
- Six stable derivatives commercialized: azoxystrobin, kresoxim-methyl, metominostrobin, trifloxystrobin, picoxystrobin and pyraclostrobin
- All have a broad spectrum activity against plant pathogenic fungi
- Do show different levels of activity against various fungal plant diseases

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Biopesticides: State of the Art and Future Opportunities

James N. Seiber,*,† Joel Coats,‡ Stephen O. Duke,§ and Aaron D. Gross#

- Reduced toxicity to non-target organisms
- Reduced persistence in the environment
- Usable in organic agriculture
- Low mammalian toxicity
- Safe for farmworkers and nearby residents
- "Green" technology
- Nonrestricted use

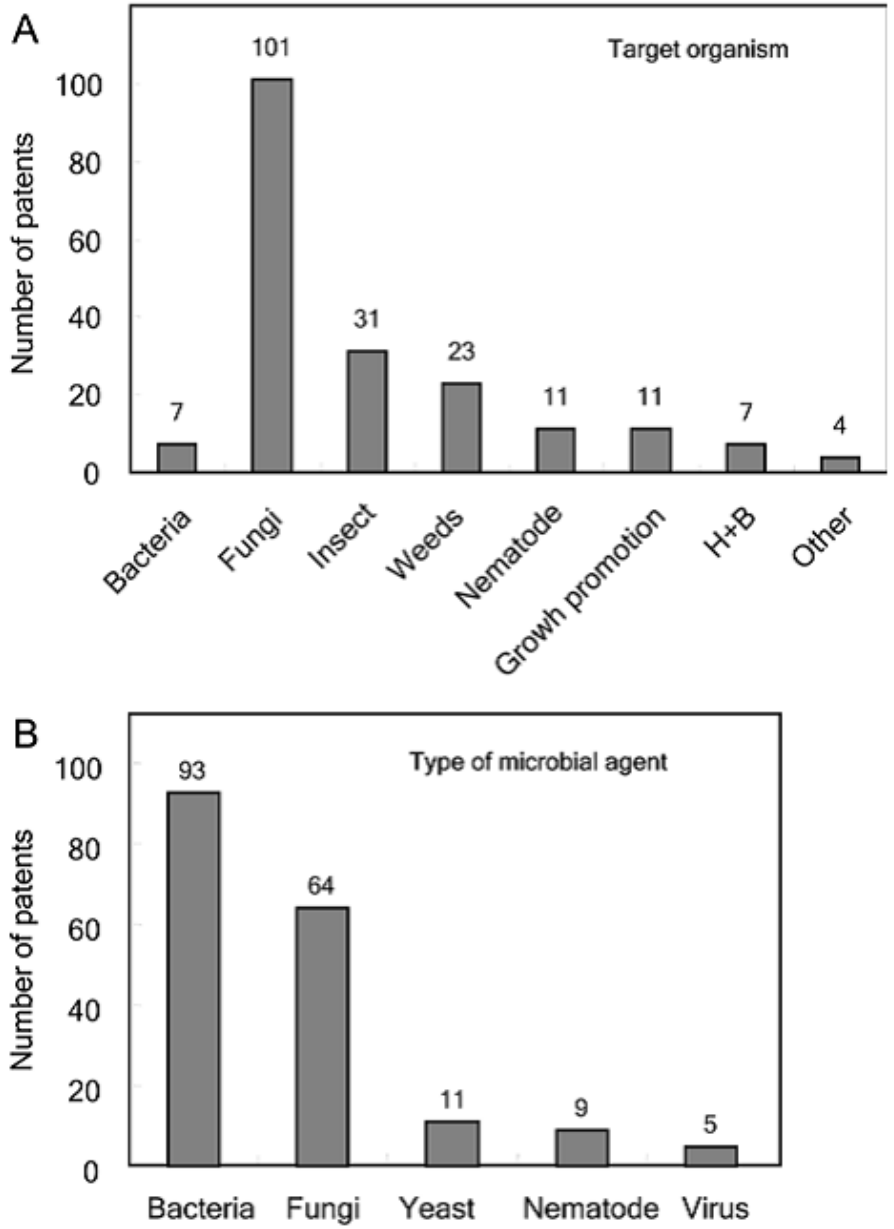


Fig. 3 Distribution of microbial pesticide patents according to the nature of the target organism (**A**) and the type of microbial agent (**B**)

Emilio Montesinos Int Microbiol (2003) 6: 245–252

Review

Trends in Biotechnology May 2012, Vol. 30, No. 5

Cell PRESS

Have biopesticides come of age?

Travis Glare[1], John Caradus[2], Wendy Gelernter[3], Trevor Jackson[4], Nemat Keyhani[5], Jürgen Köhl[6], Pamela Marrone[7], Louise Morin[8] and Alison Stewart[1]

Table I. Estimated total global market for all types of biopesticides (including biochemicals), through 2014 ($US million) [57]

Product	2008	2009	2014	CAGR [b] % 2009–2014
Bacteria	893.6	1194.3	2516.5	16.1
Fungi	113.3	150.6	288.9	13.9
Predators	98.4	128.0	247.2	14.1
Virus	60.1	80.0	155.5	14.2
Others [a]	34.6	47.1	91.9	14.3
Total	1200.0	1600.0	3300.0	15.6

[a]Includes protozoa, nematodes.

[b]Compound Annual Growth Rate.

- At least 140,000 chemicals to find one commercially acceptable synthetic pesticide
- Cost is > $250 million and takes 10 years
- Cost to develop a biopesticide is $ 3–5 million and takes approximately 3 years to get to market in the USA

microbial biotechnology

Microbial Biotechnology (2010) **3**(5), 544–563 doi:10.1111/j.1751-7915.2010.00179.x

Abdessamad Debbab,[1] Amal H. Aly,[1] Wen H. Lin[2] and Peter Proksch[1]*
[1]*Institut für Pharmazeutische Biologie und Biotechnologie, Heinrich-Heine Universitaet Duesseldorf, Germany.*
[2]*National Research Laboratories of Natural and Biomimetic Drugs, Peking University, Health Science Center, 100083 Beijing, People's Republic of China.*

Minireview

Bioactive Compounds from Marine Bacteria and Fungi

REVIEW

Folia Microbiol. **48** (5), 563–571 (2003) **http://www.biomed.cas.cz/mbu/folia/**

Alternative Sources of Biologically Active Substances

V. B HAL

Institute of Microbiology, Academy of Sciences of the Czech Republic, 142 20 Prague, Czechia
e-mail behal@biomed.cas.cz

Received 16 June 2003

ABSTRACT. The majority of antibiotics and substances with diverse biological activity used in medicine are produced by actinomycetes, nonfilamentous bacteria and fungi. Other microorganisms, such as myxobacteria, pseudomonads, nocardias, basidiomycetes, marine microorganisms, enterobacteria, halobacteria, hyperthermophiles *etc.* are investigated for new biologically active metabolites.

Review Microbes and Infection 5 (2003) 535–544

Endophytes as sources of bioactive products

Gary A. Strobel *

Appl Microbiol Biotechnol (2015) 99:1571–1586
DOI 10.1007/s00253-014-6334-y

MINI-REVIEW

Antimicrobial compounds from seaweeds-associated bacteria and fungi

Ravindra Pal Singh · Puja Kumari · C. R. K. Reddy

frontiers in MICROBIOLOGY

REVIEW ARTICLE
published: 27 March 2013
doi: 10.3389/fmicb.2013.00065

The diversity of anti-microbial secondary metabolites produced by fungal endophytes: an interdisciplinary perspective

Walaa Kamel Mousa[1,2] and Manish N. Raizada[1]*

[1] *Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada*
[2] *Department of Pharmacognosy, Mansoura University, Mansoura, Egypt*

Fence Row Farming – Improving Soil Processes

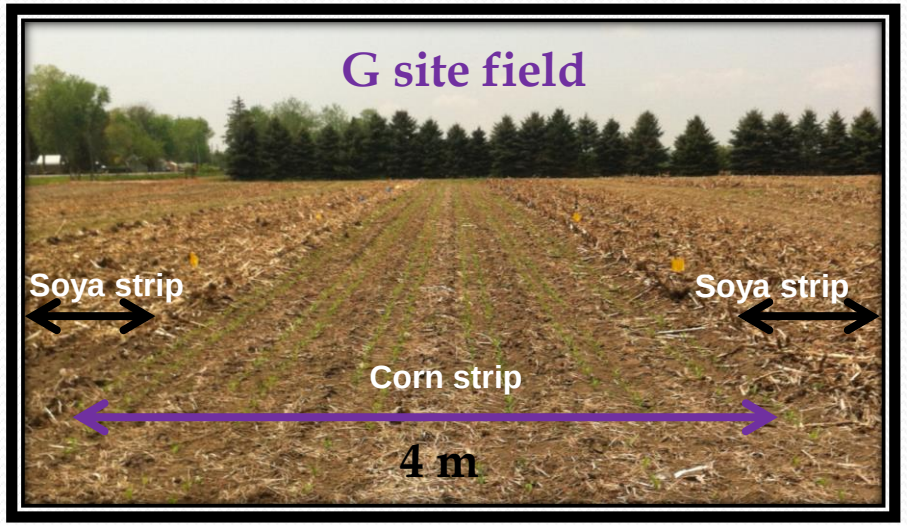
Mr. and Mrs. Dean Glenney, Dunnville, Ontario

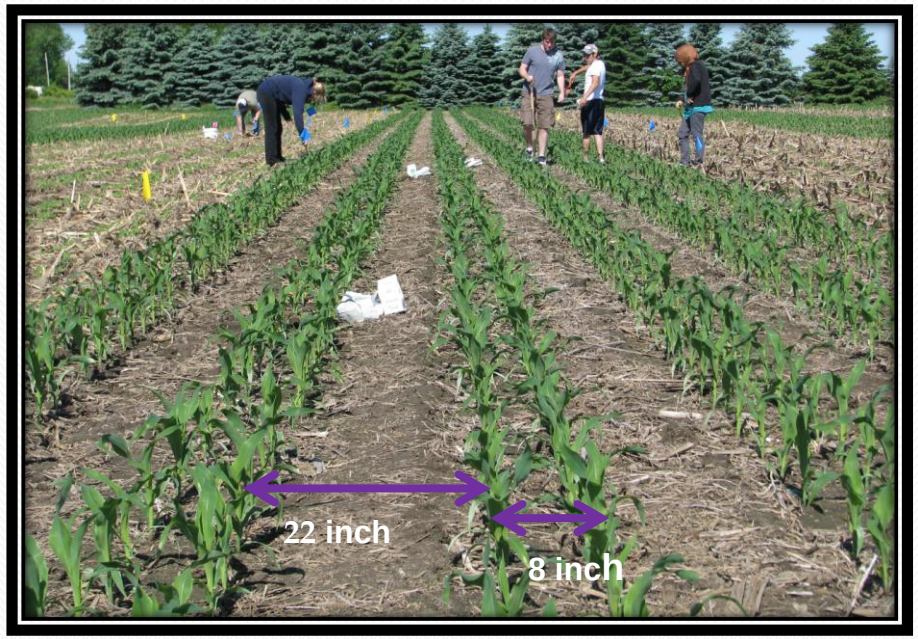
Average Corn Yield at 301 bu/acre for corn and 62bu/acre soybeans; yields 2X times that of the county average

Identification of physical, chemical and biological factors involved in corn productivity

High yielding G site corn production field

No-till strip row farming practice



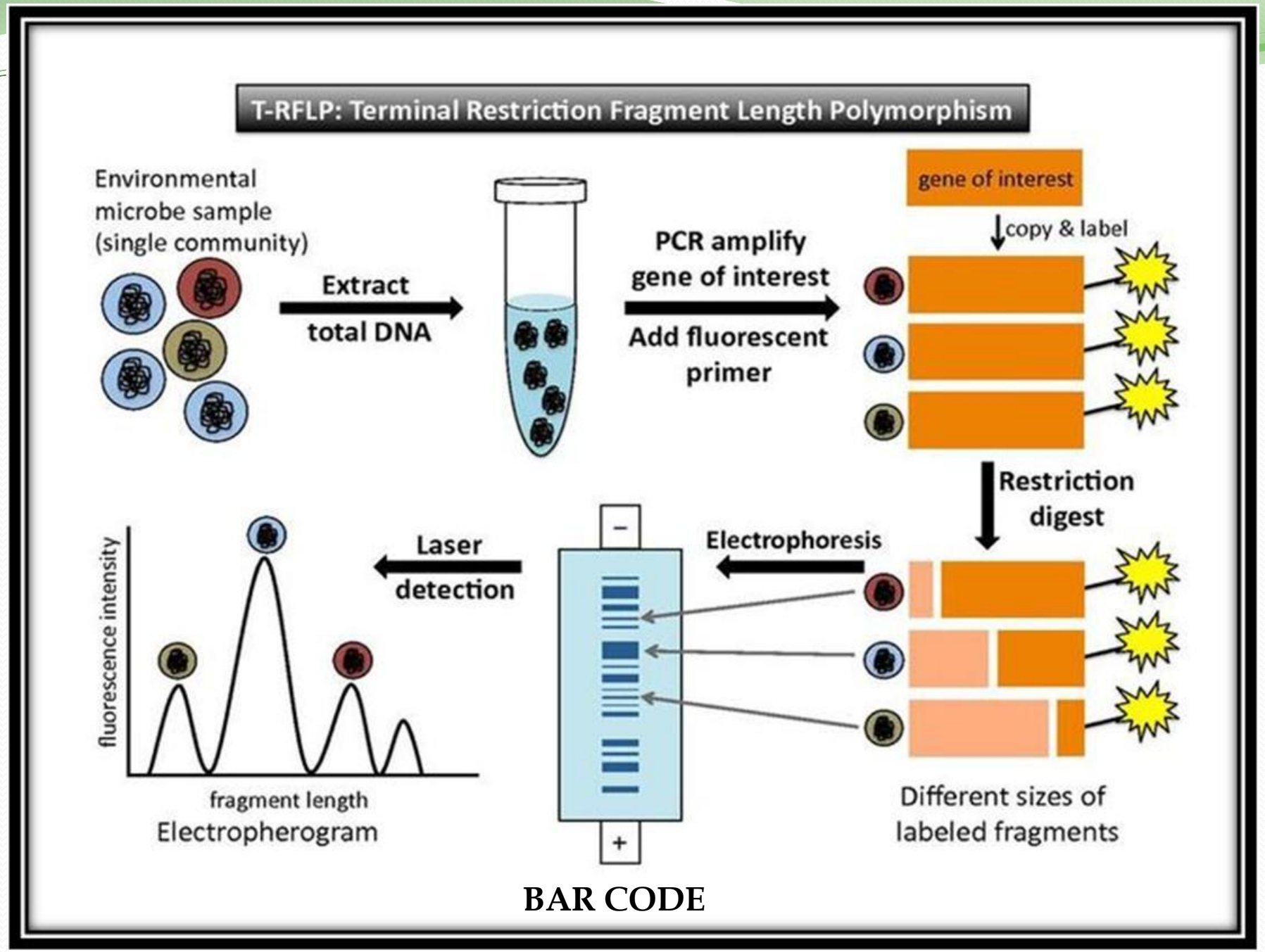


Conventional field (H site)

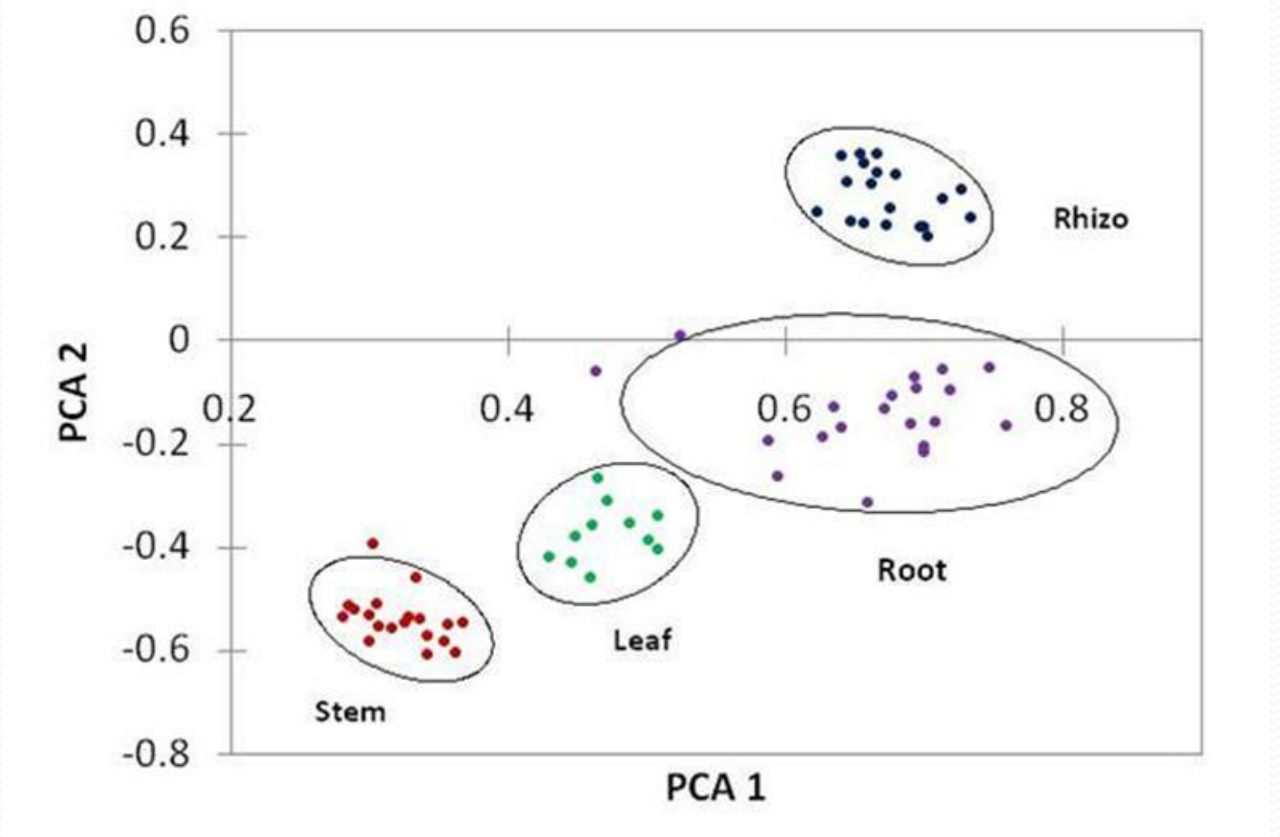
Harvested corn ears from G and H sites

G site ear

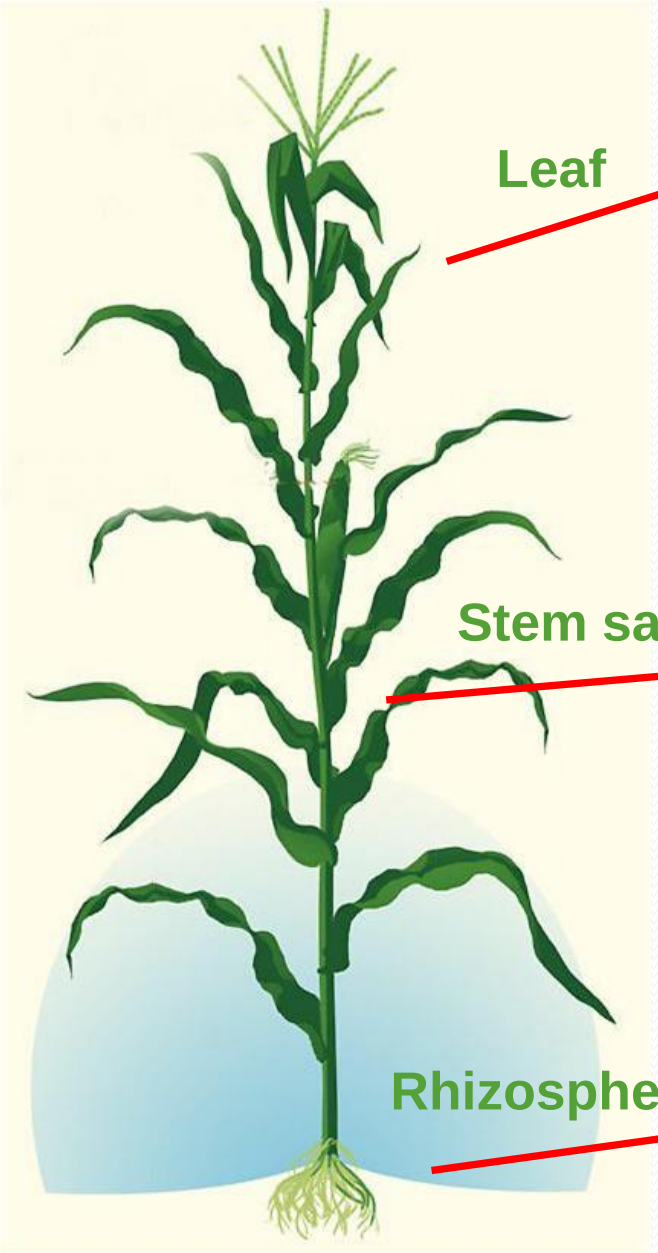
H site ear

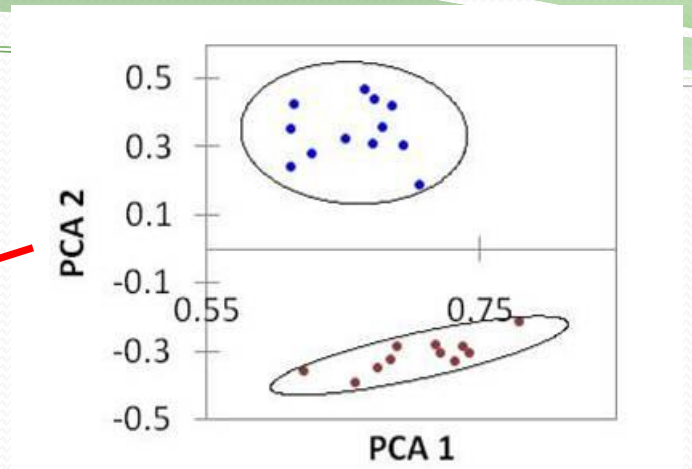

T-RFLP: Terminal Restriction Fragment Length Polymorphism
Environmental microbe sample (single community)
Extract total DNA
PCR amplify gene of interest
Add fluorescent primer
gene of interest
copy & label
Restriction digest
Electrophoresis
Laser detection
fluorescence intensity
fragment length
Electropherogram
Different sizes of labeled fragments
BAR CODE

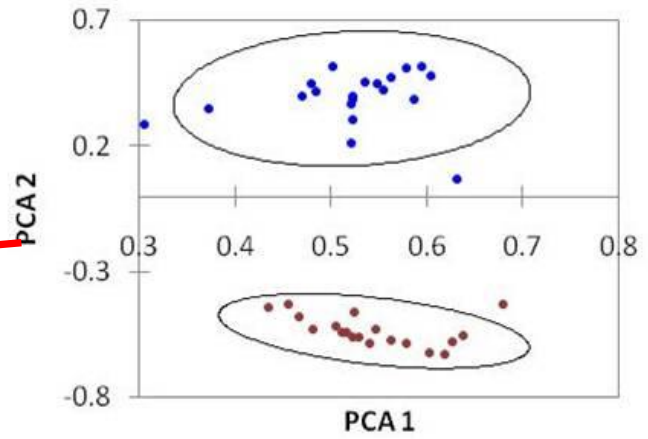
Bacterial diversity analysis using TRFLP technique

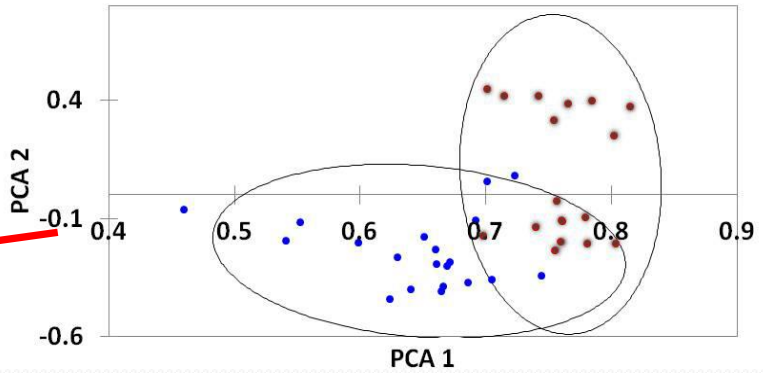


TRFLP of bacteria populations in various corn tissues of 20 plants sampled from a high yielding soil at 60 days (V10) after planting.







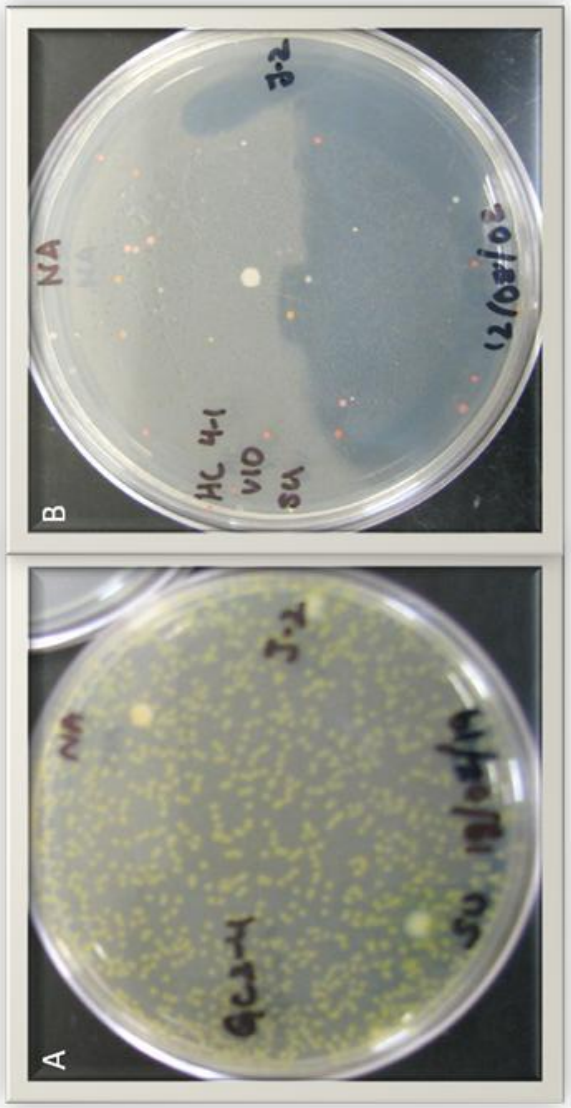


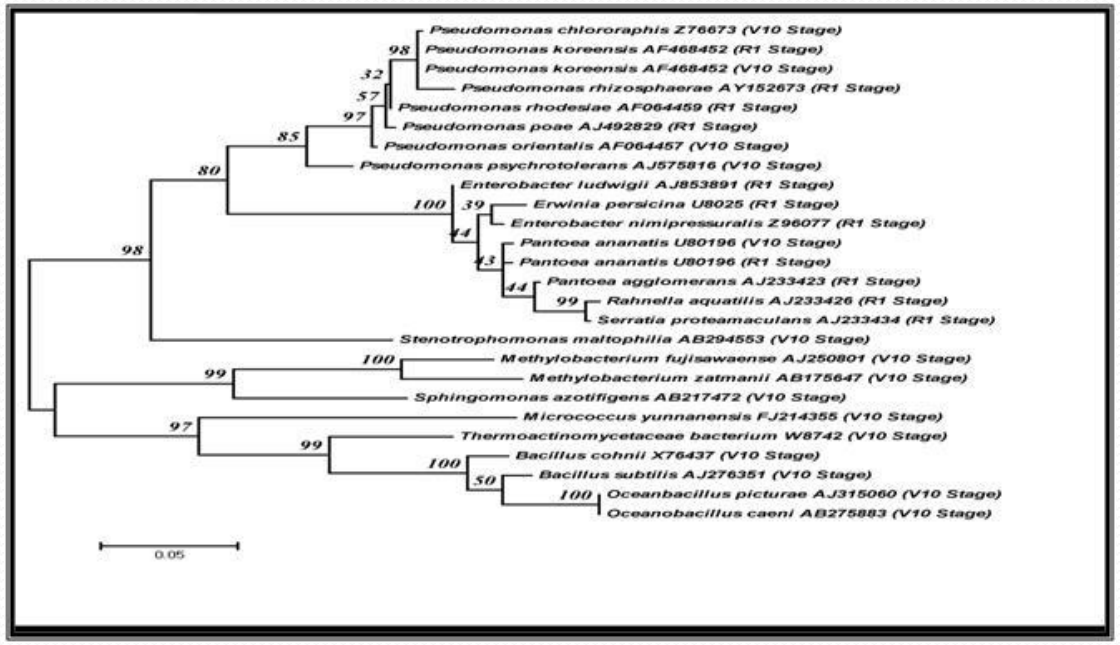
● = 150 Bu/A

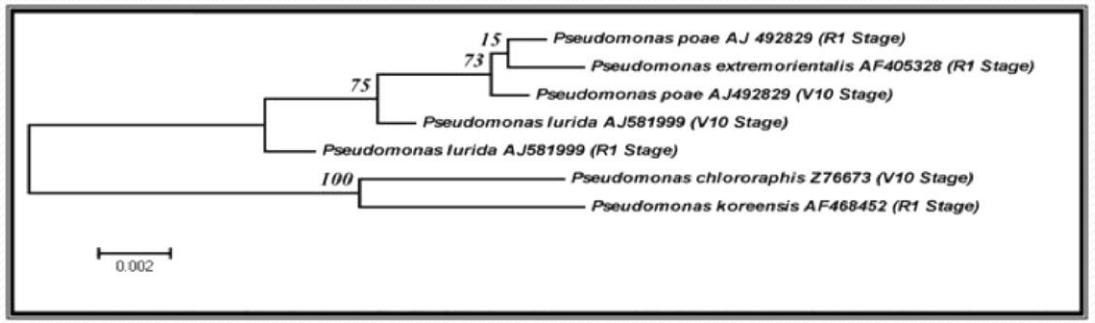
● = 300 Bu/A

Comparison of bacterial TRFLP profiles of 20 corn plants harvested from a high and average production site at 60 days (V10) after planting

Bacteria isolated from stem sap of corn plants from G and H sites at V10 growth stage.







Biologisk

Disease Suppressive Soils: Pathogen is present but no disease occurs.

Eur J Plant Pathol
DOI 10.1007/s10658-007-9201-1

FULL RESEARCH PAPER

Management of resident plant growth-promoting rhizobacteria with the cropping system: a review of experience in the US Pacific Northwest

R. James Cook

Fig. 1 Views of the same area within a 1-ha experimental plot cropped to continuous monoculture wheat starting in 1967/68 crop year. *Left*, 1974, the 7th year of monoculture facing north, showing the response to chloropicrin fumigation. *Right*, 1982, the 15th year of monoculture wheat, facing south but otherwise the same area within the 1-ha plot, with the man standing on the border separating a subplot fumigated from an adjacent subplot not fumigated. Yield of wheat in the non-fumigated plots was roughly 50% of the yield in fumigated plots in the 7th year of monoculture and 95% of the yield in fumigated plots in the 15th year of monoculture

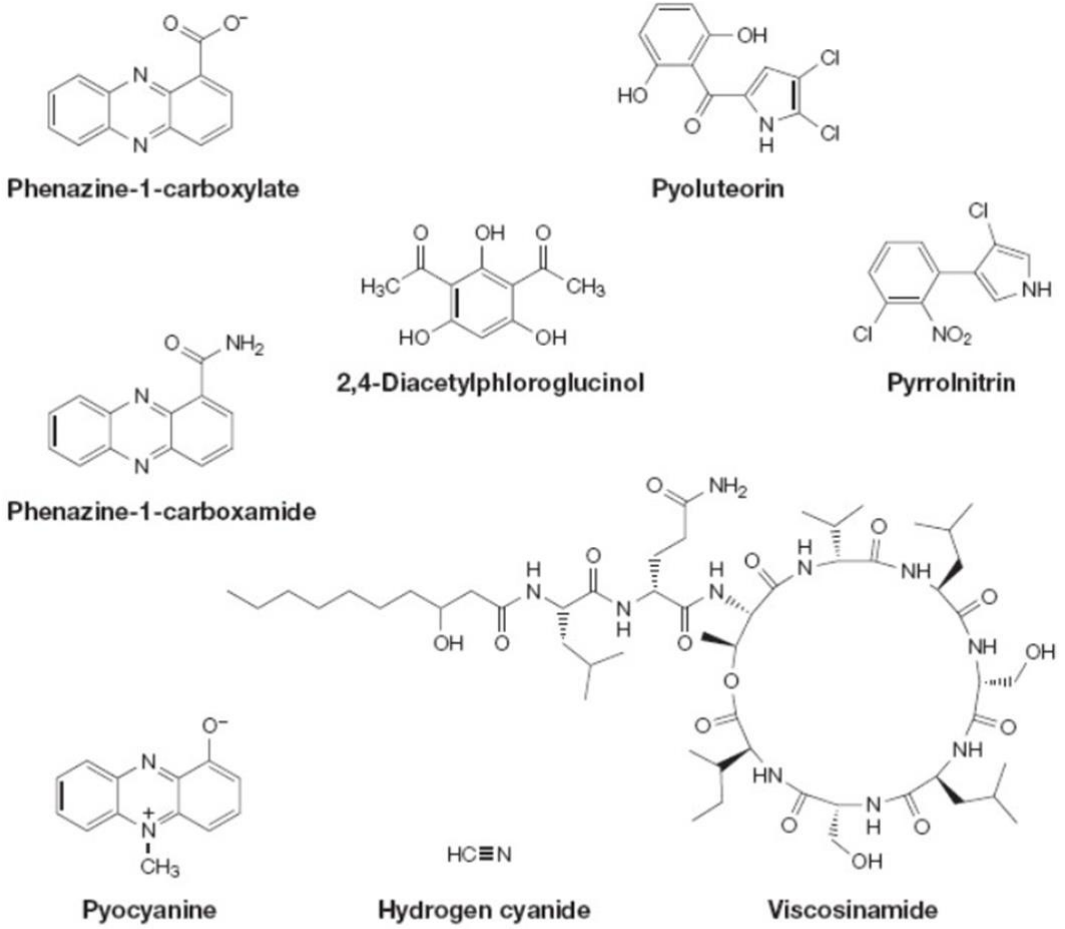
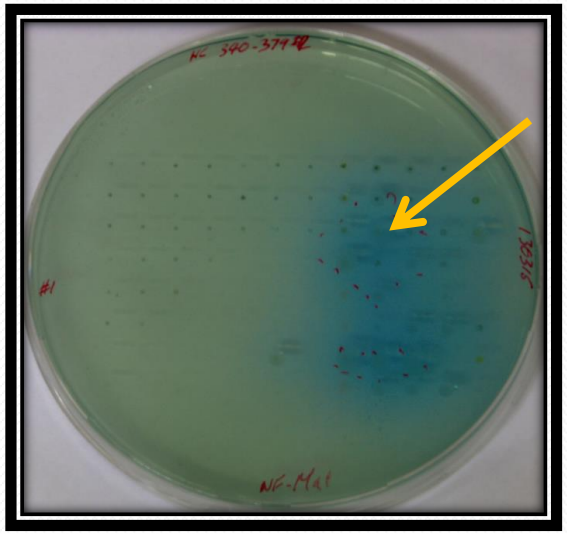
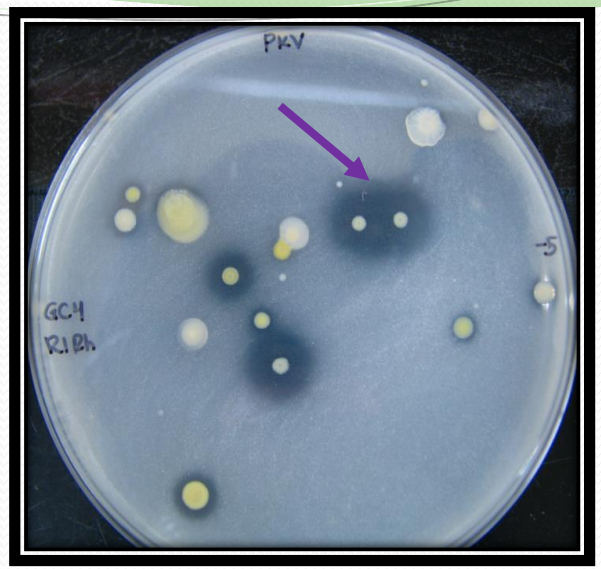


Figure 2 | **The antibiotic compounds produced by fluorescent pseudomonads that are relevant for biocontrol.** The phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin and cyclic lipopeptides are all diffusible, whereas hydrogen cyanide is volatile.

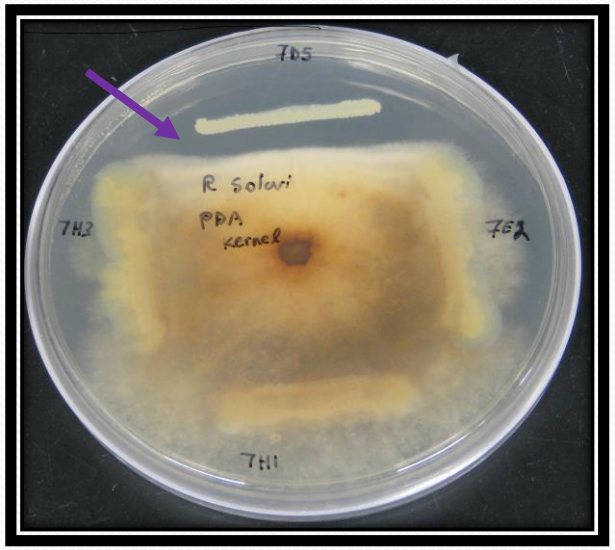
WHAT ARE THESE HEALTHY FUNCTIONS



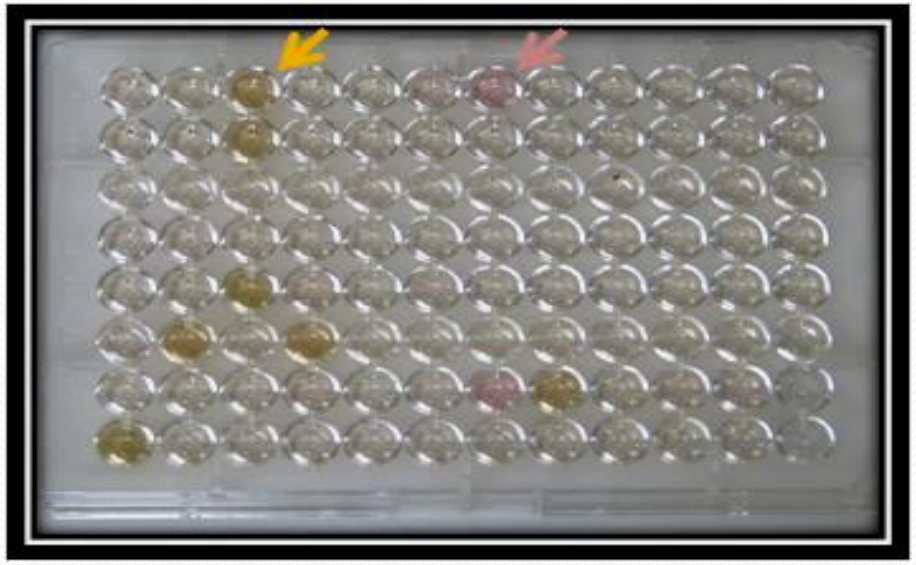
N fixing activity



P:K:Mg solubilization



Antifungal activity



Hormone (IAA) production

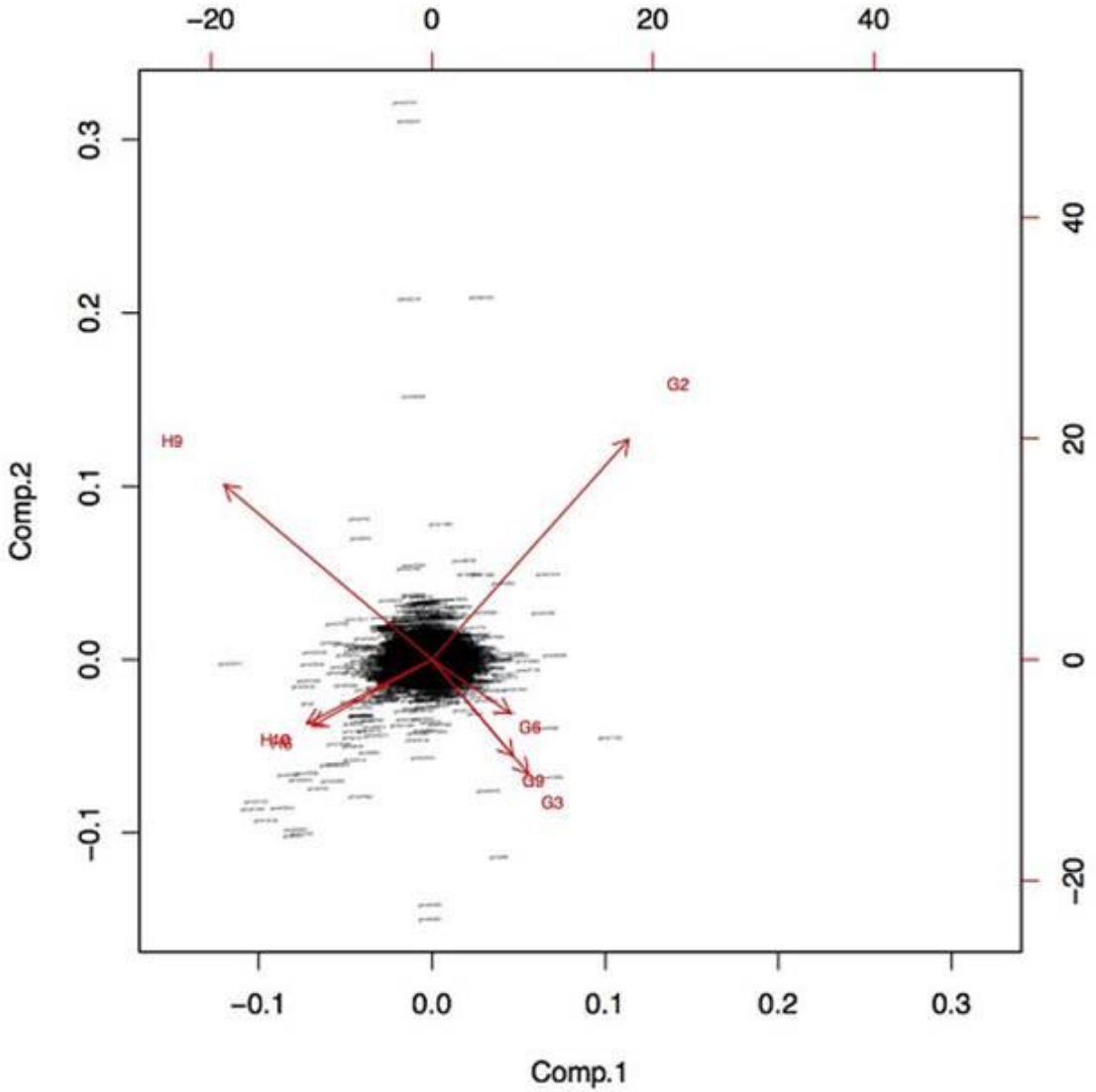


Figure 2. Compositional PCA Biplot comparing G and H samples. The first component (38% of the variance) separates G and H samples into their respective groups. Component 2 represents a further 32% of the variance. Sample H1 was removed as it was an outlier (Figure 1).

ITS ALL ABOUT FUNCTION

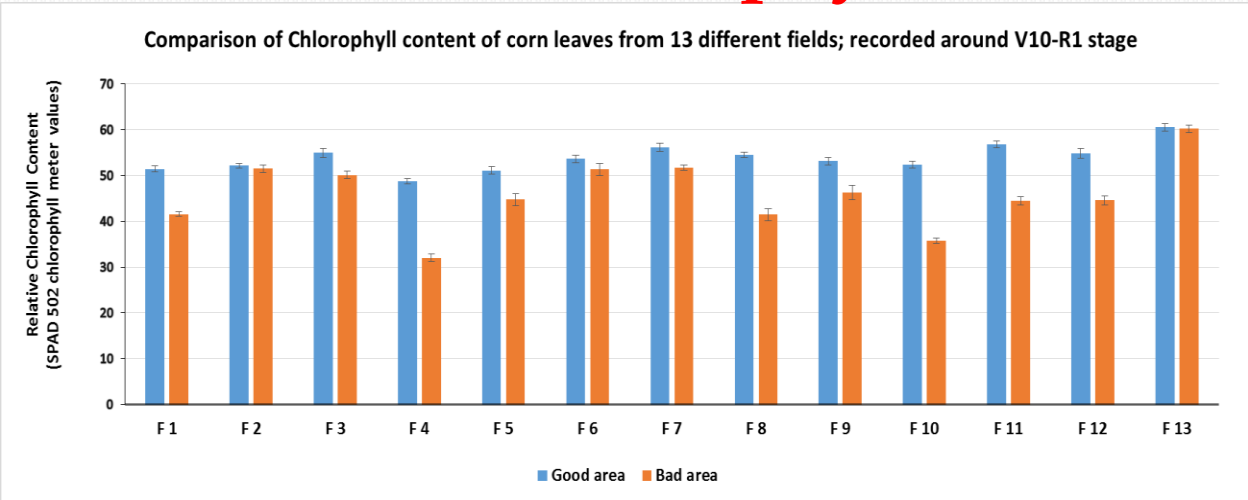
- RNA analysis will tell us what functions are impacting plant performance
- 2.5 billion amplicons identified from corn sap differentiated plants grown in soils with high and average yields

NORMALIZED DIFFERENCE VEGETATION INDEX (NDVI)

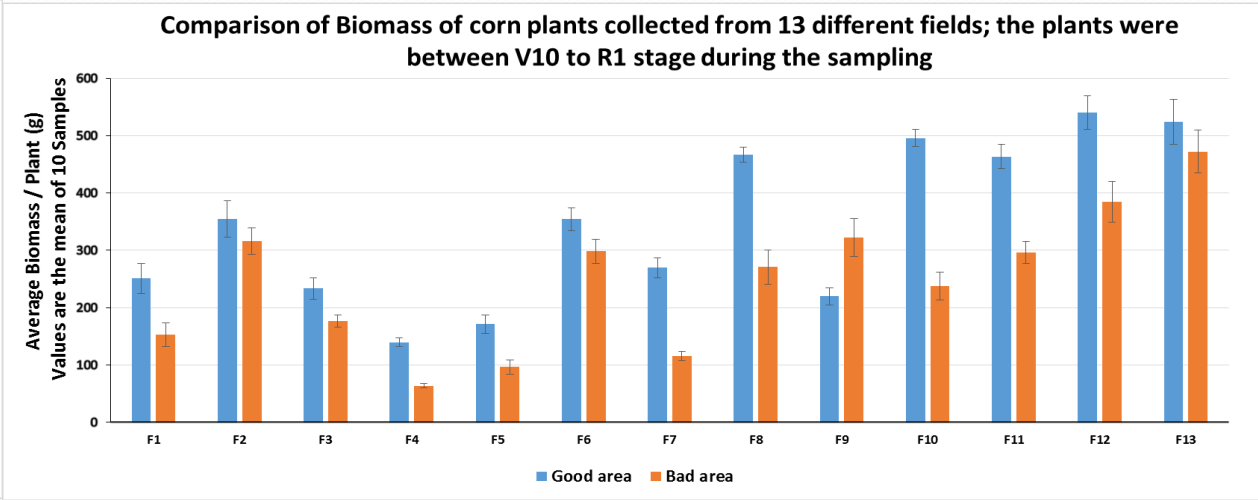
EVEN SMALL PLOTS HAVE ENORMOUS VARIABILITY AMONG THE PLANTS (UofG long term rotations)

BIOMETRIC MEASUREMENTS OF CORN PLANTS FROM HEALTHY AND STRESSED SITES OF 13 DIFFERENT FIELDS

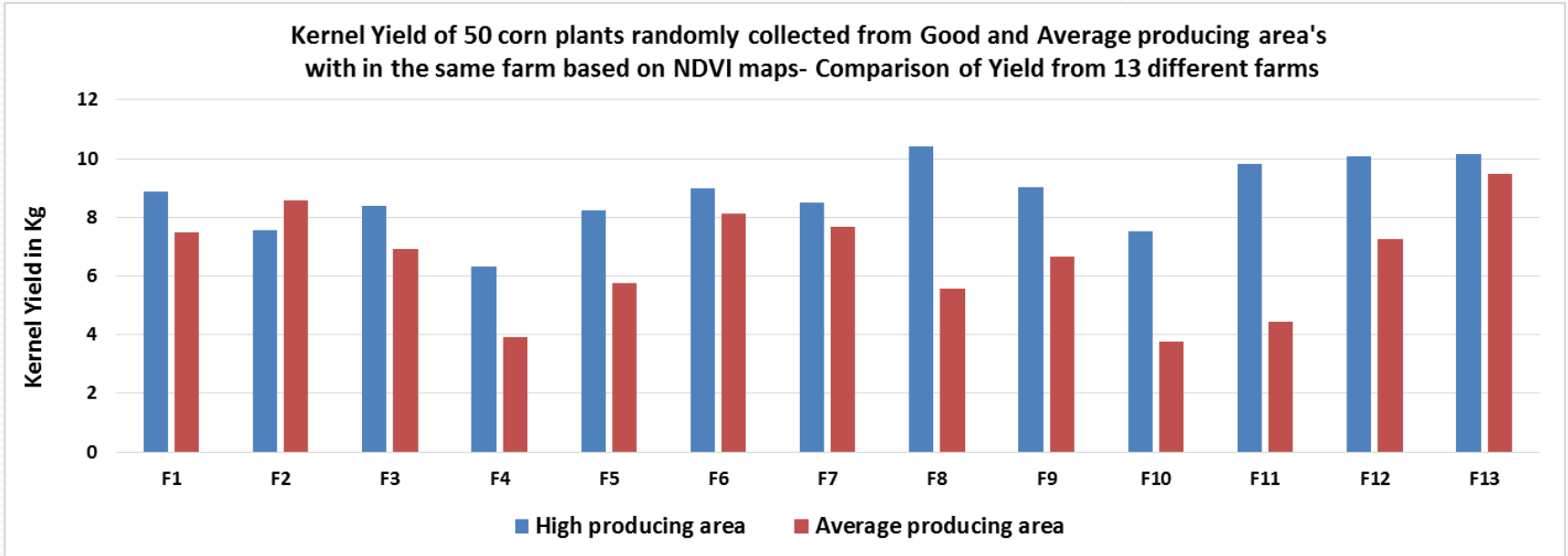
Chlorophyll



Plant Biomass



Yield



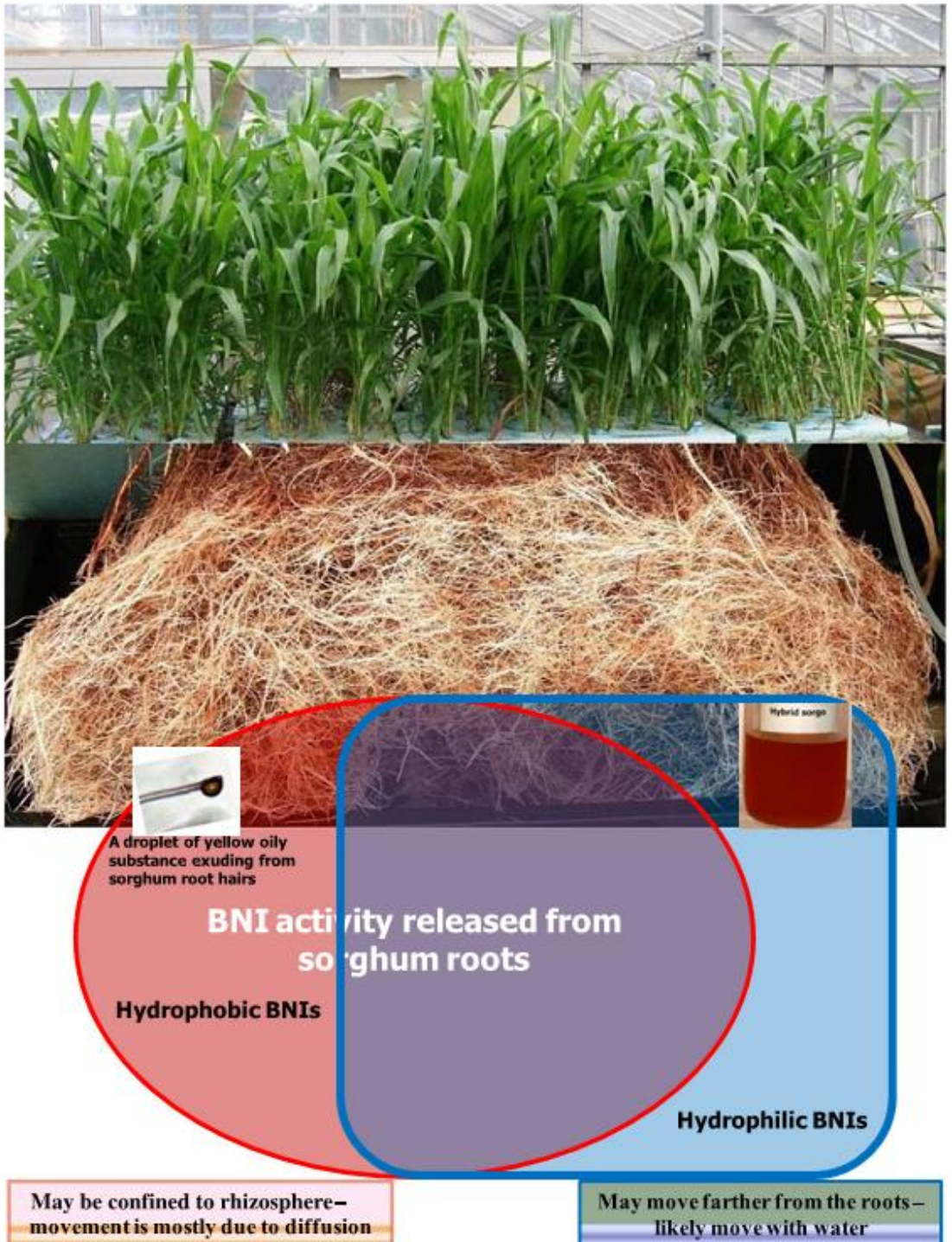
AGRICULTRUAL PRODUCTION SITES

- High correlations between yields and aerial monitoring
- Most fields have 1/3 high, 1/3 average and 1/3 poor yield potential
- Focusing on high versus low will allow for identification of drivers for crop yield

THE MOST O REA FOR FUTUR TS

Fig. 1 Hydrophobic- and hydrophilic-nitrification inhibitors (BNIs) released from sorghum roots and its significance to BNI function

Subbarao et al Plant & Soil 2012



Billions of horticultural plants are now grafted to take advantage of more resilient root systems

YIELD RESPONSES OF GRAFTED TOMATO PLANTS

Rootstock	Yield 2011- 2012 Tons per acre	Percent Increase Vs control
Control	31.9	
Beaufort	53.7	68.3
Bruce	46.2	44.8
RST-04-105-T	55.8	**74.9**
Survivor	38.5	20.7
P279	46.6	46.1
Q183	44.0	38.0

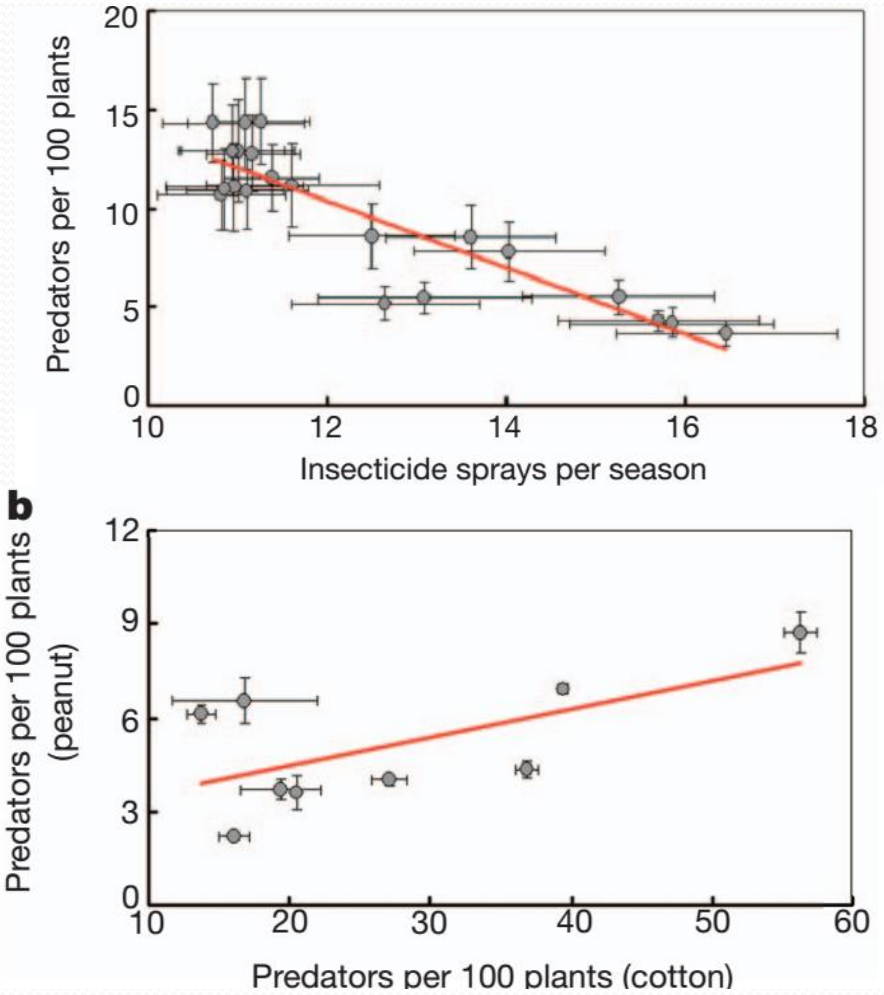
IF WE CAN GET BETTER ROOTS TO OUR PLANTS WITHOUT GRAFTING

> $2000 per A

Steve Loewen - U of G, Amy Turnbull – Fanshawe College

Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services Yanhui Lu et al (2012)

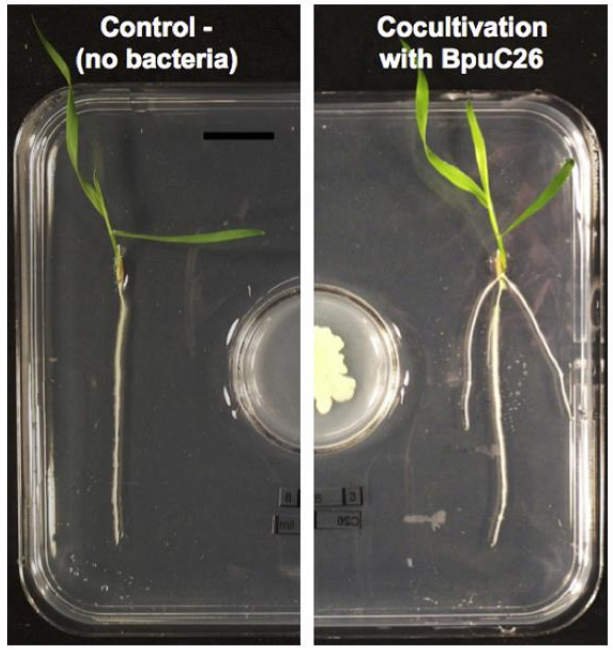
- After 20 years of growing crops producing Bt insecticides significant reduction were seen with major insect pests
- This was due to the great increase in biological control due to the increase in ladybirds, lacewings, spiders etc.
- biocontrol services spilled over onto neighbouring crops of maize, peanut, soybean etc



Plant biostimulants: Definition, concept, main categories and regulation.

P. du Jardin (2015). Sci. Hortic. http://dx.doi.org/10.1016/j.scienta. 2015.09.021

- biostimulants promote plant growth but not as fertilizers or pesticides
- Include bacteria and fungi; free-living, rhizospheric or endosymbionts
- May increase nutrient use efficiency or provide new routes of nutrient uptake e.g. mycorrhizal and non-mycorrhizal fungi, bacteria (Rhizobium) and PGPR

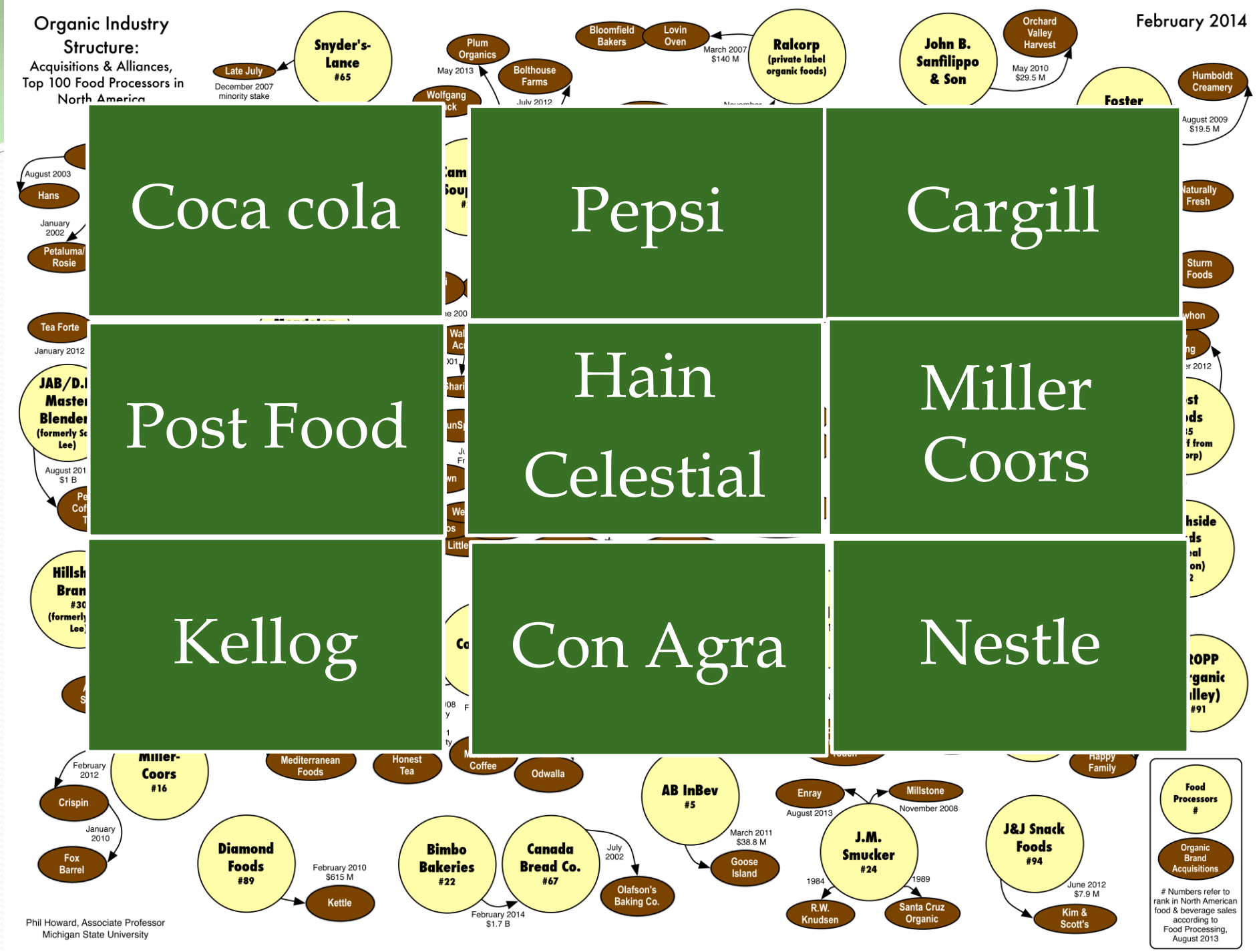


Sterile potato plants inoculated with Burkholderia phytofirmans.

Stimulation of root formation of Brachypodium by volatile compounds emitted by a PGPR *Bacillus pumilus*

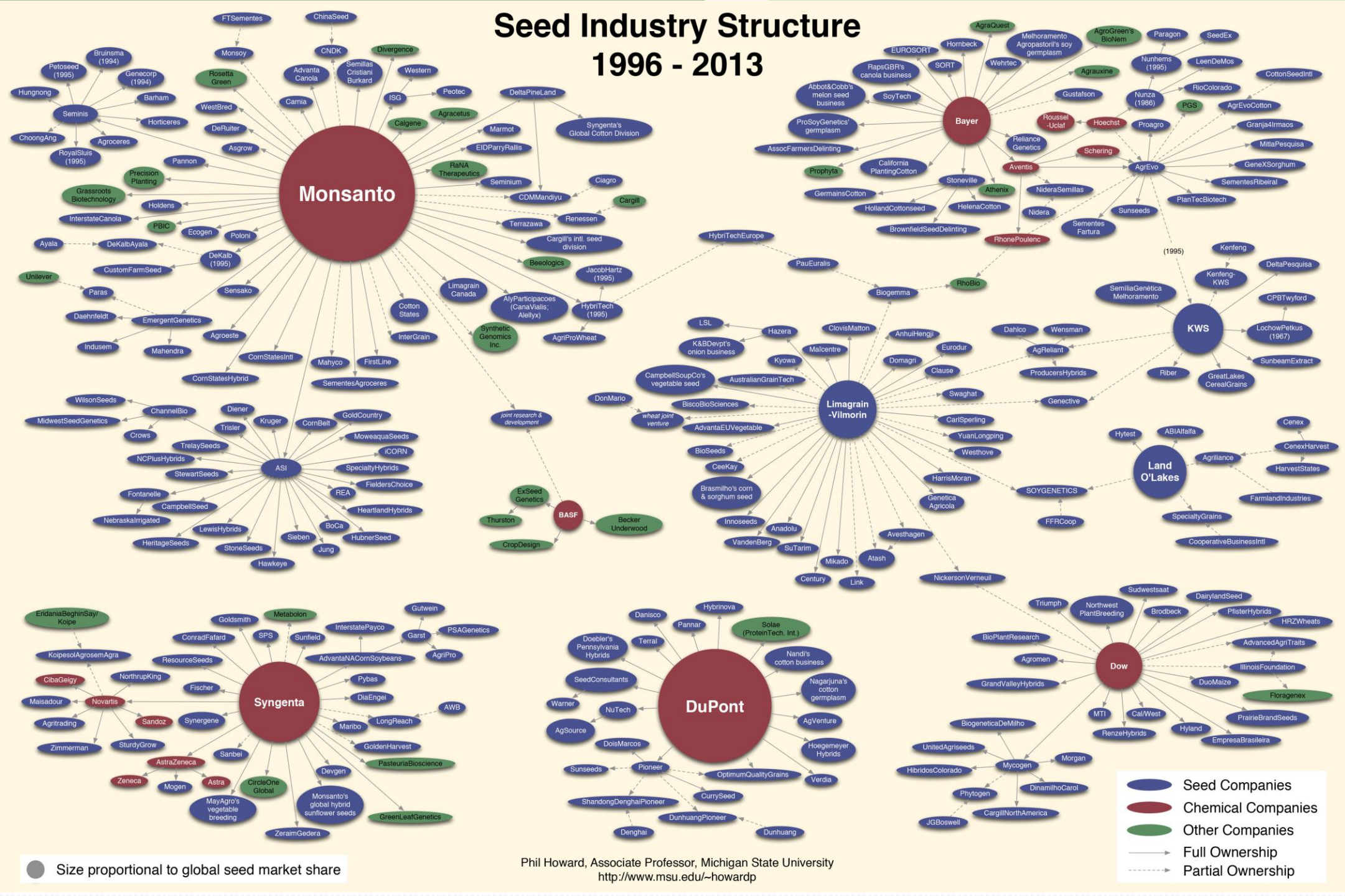
BIOLOGICAL CONTROL IS NOW A BIG BUSINESS

- 2012 Bayer purchased Biocontrol Company AgraQuest, ($500 million).
- 2012 Syngenta acquired Pasteuria Bioscience Inc ($118 million).
- 2013 Monsanto and Novozymes form the The BioAg Alliance to develop new microbial solutions to transform agriculture ($500 million) using microbial-based solutions



In 1995 there were 81 independent organic processing companies in the USA. By 2005, Big Food had gobbled up all but 15.

http://www.cornucopia.org/who-owns-organic/
Philip H. Howard



Monsanto, DuPont and Syngenta now control over half the global market of seed and biotech companies

Dr. Phil Howard

http://www.cornucopia.org/seed-industry-structure-dr-phil-howard/

The Next Green Revolution will Emerge from Underground

Acknowledgements

A&L Biologicals Collaborators

Research Scientists: **Dr. Soledad Saldías, Dr Salah Khabbaz, Dr. Shimaila Ali, Dr. Saveetha Kandasamy, Dr. Rafiq Islam**

Technical Assistants: Ms. Magda Konopka, Mr. Jae-min (Joseph) Park, Ms. Kristen Delaney

Coop Students: Ms. Gabrielle Zieleman, Ms. Ashley Grant, Ms. Stephanie Kerkvliet, Ms. Mallory Wiggans, Ms. Kathleen Meszaros, Ms. Kelsey MacEachern

Western Collaborators

Dr. Greg Gloor, Dr. Jean M Macklaim

AAFC Collaborators:

Dr. Ze-Chun Yuan, Mr. Brian Weselowski

Grower Collaborators:

Mr. Dean Glenney, Mr. Clarence Hessels, Mr. Jim Campbell, Mr. Jeff Bloch, Dr. Bill Deen, Mr. Shane VandenDries, Mr. Robert Koch, Mr. Jeff Ferguson, Mr. Brent Pilkington, Mr. Tony Balkwill, Mr. Shantz SchumHaven